

Remarks

Claims 22-45 are pending in the subject application. By this Amendment, Applicants have amended claims 22, 28, 30, 34, 41, and 43, canceled claims 27, 29, 31, 36-40, 42, and 44, and added new claim 46. Support for the new claim and amendments can be found throughout the subject specification and in the claims as originally filed. Support for the amendment to claim 30 clarifying that step (ii) is carried out by measuring energy transfer between the fluorescent molecule and the bound label can be found, for example, at page 6, last paragraph, of the subject specification. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 22-26, 28, 30, 32-35, 41, 43, and 46 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

The disclosure in the subject application is objected to because of informalities. Specifically, the Examiner has indicated that the designation for the sequence identifiers at pages 12 and 13 of the subject specification is improper. In accordance with the Examiner's suggestion, the specification has been amended to recite "SEQ ID NO:" instead of "SEQ ID NO." or "SEQ ID No." Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claims 22-35, 40, and 41 are rejected under 35 USC §112, second paragraph, as indefinite. Applicant asserts that the claims are definite. However, by this Amendment, Applicant has amended the claims in order to lend greater clarity to the claimed subject matter. Applicant gratefully acknowledges the Examiner's helpful suggestions regarding acceptable claim language. Claim 22 has been amended to delete the language "capable of" and to include a step for detecting conformational changes in an enzyme in the claimed method. Applicant has also amended claim 22 to specify that the target polynucleotide is not labeled. Support for the amendment to step (ii) in claim 22 can be found, for example at 3, lines 11-12, of the subject specification. In addition, support for the amendment of claim 22 which specifies that the enzyme is labeled with a fluorescent molecule that has a modifiable signal, and that the target polynucleotide is not labeled, can be found throughout the specification, including, for example, at page 3, lines 33-35, and page 7, paragraph 2, and in the Example at pages 10-13 of the subject specification. Applicant has also amended claims 28 and 41 to delete reference to the phrase "capable of" and has canceled claim 40. Accordingly,

reconsideration and withdrawal of the rejection under 35 USC §112, second paragraph, is respectfully requested.

Claims 22, 23, and 25 are rejected under 35 USC §102(b) as anticipated by Holzrichter *et al.* (WO 95/06138). The Examiner indicates that the Holzrichter *et al.* reference teaches a method of determining the sequence of a polynucleotide wherein the enzyme is a polymerase and is immobilized on a solid support. Applicant respectfully traverses this ground of rejection.

Applicant respectfully asserts that the Holzrichter *et al.* reference does not anticipate the claimed invention. The Holzrichter *et al.* reference does not disclose an enzyme comprising a bound fluorescent molecule. Rather, in the Holzrichter *et al.* reference, the fluorescent molecule is on the polynucleotide. Applicant's claimed invention specifies that the enzyme comprises a fluorescent molecule bound thereto. As the Examiner is undoubtedly aware, in order to anticipate, a single reference must disclose within the four corners of the document each and every element and limitation contained in the rejected claim. *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991). Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(b) is respectfully requested.

Claims 22-27, 29, 31, 32, 34, and 36-45 are rejected under 35 USC §102(e) as anticipated by Chan (U.S. Patent No. 6,210,896). In addition, claims 28, 30, and 35 are rejected under 35 USC §103(a) as obvious over Chan (U.S. Patent No. 6,210,896) in view of Ha *et al.* (1999). The Examiner asserts that the '896 Chan patent teaches a method of determining the sequence of a polynucleotide, comprising the steps of reacting a target polynucleotide with an enzyme that is capable of interacting with and processing along the polynucleotide, under conditions sufficient to induce enzyme activity; and detecting conformation changes in the enzyme and wherein the enzyme contains a detectable label. The Examiner further asserts that the Ha *et al.* reference teaches the use of fluorescence polarization anisotropy to observe conformational fluctuations and catalytic reactions of an enzyme at single molecule resolution. As noted above, the rejections of claims 27, 29, 31, 35-40, 42, and 44 are moot in view of their cancellation. Applicant respectfully traverses both the §102(e) and §103 rejection together for the remaining rejected claims.

Applicant respectfully asserts that the cited references do not teach or suggest the claimed invention. The disclosure in the '896 Chan patent requires a labeled polymer molecule, *i.e.*, a target

polynucleotide, and the interaction between the labeled polymer and the labeled enzyme is detected. The method of the subject invention does not require a labeled polymer. Thus, Applicant respectfully asserts that the '896 Chan patent does not anticipate or render the claimed invention obvious. Amended claims 41, 43, and 46 are not anticipated because the '896 Chan patent only discloses enzymes on a solid support that are labeled with one half of a FRET pair. There is no disclosure in the '896 Chan patent of a solid support comprising an enzyme that is labeled with both halves of a FRET pair. Support for the amendment to claim 41 can be found, for example, at page 5, line 33, and page 6, paragraph 2 of the subject specification. Reference to the use of FRET labels can be found, for example at page 6, last paragraph, and the Example at pages 10-13 of the specification.

Applicant's claimed invention requires only a simple measure of fluorescence of a single molecule in order to determine the sequence of a polynucleotide. There is no need for incorporated nucleotides to be labeled. The method therefore avoids problems associated with distinguishing multiple labels, for example when nucleotides are fluorescently labeled, and inhibition of enzyme activity caused by bulky labels on the nucleotides. A key requirement of the Chan patent is that a label must be present on the enzyme and a label must be present on the target polymer. Applicant's claimed method does not require that the target polynucleotide be labeled; claim 22 specifically recites that the target polynucleotide is not labeled. In addition, there is no teaching or suggestion that the conformational change of an enzyme can be detected using only a fluorescent label on the enzyme, to determine the sequence of a target polymer.

The Ha *et al.* reference is even less relevant as it is not concerned with DNA sequencing procedures but is seeking to study protein folding. The general teaching of Ha *et al.* is that a fluorescent label can be used to monitor protein structure at single molecule resolution. The enzyme studied, staphylococcal nuclease, is used only as a model protein with no teaching or suggestion to apply this technique in the field of DNA sequencing. Even assuming, *arguendo*, that there was a reason for the skilled artisan to combine the teachings of the Ha *et al.* reference and the '896 Chan patent, the skilled artisan would still not arrive at Applicant's claimed invention.

Accordingly, reconsideration and withdrawal of the rejections under 35 USC §§102(e) and 103(a) is respectfully requested.

Claim 33 is rejected under 35 USC §102(e) as anticipated by Chan (U.S. Patent No. 6,355,420). The Examiner asserts that the '420 Chan patent teaches a method of determining the sequence of a polynucleotide, comprising the steps of reacting a target polynucleotide with an enzyme that is capable of interacting with and processing along the polynucleotide, under conditions sufficient to induce enzyme activity; detecting conformation changes in the enzyme and wherein the enzyme contains a detectable label; and detection by fluorescence imaging. Applicant respectfully traverses this ground of rejection.

Applicant respectfully submits that the '420 Chan patent does not teach or suggest the claimed invention. As noted in regard to the rejections based on the '896 Chan patent, the disclosure in the '896 and the '492 Chan patents require a labeled polymer molecule, *i.e.*, a target polynucleotide, and the interaction between the labeled polymer and the labeled enzyme is detected. The method of the subject invention does not require that the target polynucleotide be labeled and claim 22, from which claim 33 ultimately depends, recites that the target polynucleotide is not labeled. Thus, Applicant respectfully asserts that the claimed invention is not anticipated or obvious over the '420 Chan patent. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(e) is respectfully requested.

Applicant notes that Laing *et al.* (U.S. Patent No. 6,331,392) is referenced by the examiner in the Office Action but is not relied upon in a rejection. Applicant respectfully asserts the '392 patent is not relevant to the pending claims, as it appears to concern only a fluorescence anisotropy-based method of detecting the strength of binding between a target RNA molecule and a ligand. There is no disclosure of a sequencing technique.

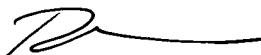
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicant's agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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